EXHIBIT 12

ORIGINAL ARTICLES

A Ferret Model of Electrical-Induction of Arterial Thrombosis that is Sensitive to Aspirin

William A. Schumacher, Thomas E. Steinbacher, John R. Megill, and Stephen K. Durham

Departments of Pharmacology and Experimental Pathology, Bristol-Myers Squibb Research Institute, Princeton, New Jersey, U.S.A.

An experimental model of acute thrombosis was developed in pentobarbital-anesthetized ferrets. A 10-min anodal electrical stimulation of 1 mA was delivered to the external surface of the carotid artery while measuring carotid blood flow (CBF). This produced an occlusive thrombus in all vehicle-treated ferrets within 41 ± 3 min with an average weight of 8 ± 1 mg ($n = 7$). These thrombi were enriched in both platelets and fibrin and were adherent at the site of transmural vascular injury as determined by light and electron microscopy. To determine the model's sensitivity to antiplatelet drugs, aspirin or a thromboxane (TxA_2) receptor antagonist (ifetroban) were administered 15 min before electrical stimulation. Thrombus weight was reduced 58% by aspirin (10 mg/kg, i.v.) and 74% by ifetroban (1 mg/kg + 1 mg/kg per hr, i.v.). Both drugs also improved CBF and decreased vascular occlusion. Ferrets were more sensitive than rats to aspirin's inhibition of collagen-induced platelet aggregation as determined ex vivo in whole blood. Separate in vitro platelet aggregation studies revealed species differences in reactivity to U-46619 (TxA_2 receptor agonist) and collagen in the order of human > ferret > rat, with relatively lesser variations in ADP responses. These studies identify the ferret as a useful species for evaluating antithrombotic drugs in a model in which aspirin is efficacious.

Key Words: Arterial thrombosis; Ferret; Platelet aggregation; Blood coagulation; Aspirin; Thromboxane inhibitor

Introduction

Coronary artery thrombosis induced by electrical stimulation was originally developed in dogs (Salazar, 1961) and was subsequently adapted to larger arteries in rats (Hladovec, 1973), rabbits (Zweifler, 1967), pigs (van der Giessen, 1989), and monkeys (Schumacher and Heran, 1989). Electrolytic injury can be delivered to either the adventitial (rats, rabbits) or intimal surface (dogs, pigs, monkeys) of the artery, and it produces an occlusive thrombus that is platelet- and fibrin-rich and has a morphology similar to that observed in human vascular disease (Romson, et al., 1980). Both antiplatelet and anticoagulant drugs interfere with the formation of these thrombi, and fibrinolytic agents effect their dissolution. Numerous modifications and refinements of the electrolytic model have been, and are being developed (Bush and Shebuski, 1990). The acute nature and high incidence of thrombosis characteristic of this technique have made it most appropriate for pharmacological studies.

We recently adapted a different thrombosis procedure, referred to as the Folts model, to the abdominal aorta of anesthetized ferrets (Gomoll et al., 1995). The Folts model involves rapid and periodic platelet accumulation in stenotic and crush-injured arteries. A myocardial ischemia-reperfusion injury model was also employed in this study, which thereby demonstrated the utility of ferrets in the concurrent evaluation of antithrombotic and anti-ischemic drugs. We have presently adapted the method of electrolytic-induced thrombosis to the carotid artery of the ferret with the intent of producing a slower developing thrombus that would be

Address reprint requests to Dr. William Schumacher, Department of Pharmacology, Bristol-Myers Squibb Research Institute, P.O. Box 4000, Princeton, NJ 08543-4000, U.S.A.

Received December 21, 1994; revised and accepted August 18, 1995.

Journal of Pharmacological and Toxicological Methods 35, 3–10 (1996)

655 Avenue of the Americas, New York, NY 10010

1056-8719/96/$15.00
SSDI 1056-8719(95)00099-2

more fibrin-dependent than the Folts model (Bush and Shebuski, 1990). To demonstrate drug efficacy in the electrolytic model, aspirin and the thromboxane receptor antagonist ifetroban (BMS-180291; Ogletree et al., 1993) were tested at doses previously shown to be sufficient in ferrets for maximal inhibition of cyclooxygenase and TxA_2 receptors. Antithrombotic activity was assessed by decreases in thrombus weight and preservation of blood flow, while ex vivo measurements of coagulation and platelet activation provided additional pharmacologic evaluations. The in vitro reactivity of human, ferret, and rat platelets to U-46619, collagen, and ADP were determined in separate experiments to better profile the ferret relative to other species.

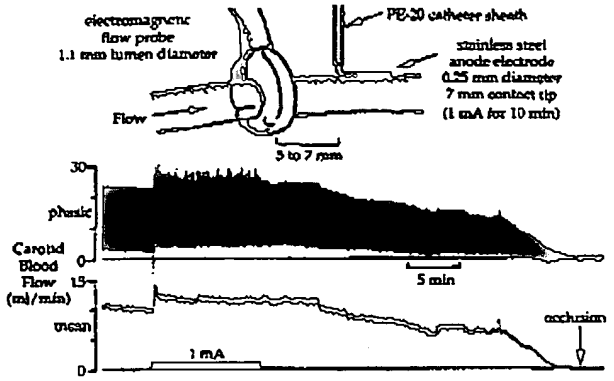


Figure 1. The experimental preparation to induce arterial thrombosis and continuous CBF tracings from a vehicle-treated ferret are shown. Occlusion occurred at 40 min after the start of stimulation, and it was sustained until 120 min, at which time a 9.4 mg thrombus was recovered from the artery.

Methods

Carotid Artery Thrombosis

Male neutered ferrets (*Mustela purotius furo*) weighing 1.1–1.3 kg were anesthetized with Na-pentobarbital (45 mg/kg i.p.). Supplemental Na-pentobarbital was administered at 20 mg, i.p., approximately once every hour (~60 mg total). Polyethylene (PE) catheters were inserted into both jugular veins (PE-90) for drug administration and into the right femoral artery (PE-50) for blood withdrawal and to monitor arterial blood pressure (ABP) with a P23Db transducer (Gould Inc., Oxnard, CA). The trachea was cannulated and mechanical ventilation was initiated using a model 665 dual phase respirator (Harvard Apparatus, South Natick, MA). The respirator was adjusted to maintain arterial $PO_2 > 80$ mm Hg and PCO_2 between 35 and 40 mm Hg as measured on an ABL 500 blood gas analyzer (Radiometer, Copenhagen, Denmark). Body temperature was monitored and maintained at 38° C with a heating pad. The right carotid artery was exposed, and a piece of Parafilm "M" (American National Can, Greenwich, CT) was inserted under the vessel for electrical isolation. A stainless steel L-shaped wire and an electromagnetic flow probe was placed on the artery and attached to a model MDL 1401 flowmeter (Skalar, Delft, Netherlands) for measurement of carotid artery blood flow (CBF) (Figure 1). A 1-mA anodal current was delivered through this wire for 10 min using a constant DC current stimulator (MOD 870; World Precision Instruments, New Haven, CT). The cathode was a alligator clip attached to the hind limb. The electrode was removed after termination of stimulation. Some preliminary experiments were also performed using 0.5-mA stimulation. CBF and ABP were monitored continuously on a R611 recorder (Sensor Medics, Anaheim, CA); however, the flow probe was switched off after ~20 min of zero flow to prevent overheating of the vessel and turned on intermittently thereafter. The preparation was monitored for 2 hr, but it was maintained for up to 3 hr if the vessel remained patent. At these times the artery was isolated over its exposed length with two microaneurysm clips and dissected free. The vessel segment was opened lengthwise under a stereomicroscope. The thrombus was removed and its wet weight was determined on a Sartorius R-160P balance (Brinkmann Instruments Inc., Westbury, NY).

Drug treatments were administered i.v. starting 15 min before electrical stimulation and consisted of vehicle ($n = 7$), aspirin (10 mg/kg; $n = 5$), and ifetroban, which was administered as a loading dose plus continuous infusion (0.3 + 0.3 or 1 + 1; mg/kg + mg/kg per hr with $n = 5$ and 7, respectively). The vehicle included warm water for aspirin (1 mL/kg) and a 10% solution of ethanol in 0.2% sodium carbonate for ifetroban (1 mL/kg + 25 μL/min). Aspirin and ifetroban were synthesized at Bristol-Myers Squibb.

Morphology

The thrombus and associated carotid arterial segment of three additional vehicle-treated ferrets were removed following occlusion for light and transmission electron microscopy, and immersed in McDowell-Trump fixative containing 3.7% formaldehyde and 1.0% glutaraldehyde in 0.1-mol cacodylate buffer per liter, pH 7.4 (McDowell and Trump, 1976). The thrombus samples for scanning electron microscopy were removed from the artery prior to immersion in McDowell-Trump fixative. All specimens were post-fixed in 1% osmium tetraoxide, and dehydrated in graded alcohols. The specimens were either embedded in epoxy resin by routine methods for light and transmission electron microscopy, or subjected to critical-point drying for scanning electron microscopy.

Clotting Times

Arterial blood was sampled before drug treatment (2 mL) and after vessel removal (3 mL) by withdrawing into a 1/10 final vol of 3.8% Na-citrate. Blood samples were centrifuged for 3 min in a Microfuge E centrifuge (Beckman, Palo Alto, CA) to obtain plasma. Clotting of fresh plasma and blood was measured at 37° C using a mechanical coagulation time (Fibrometer; Baxter Healthcare, Miami FL). The activated partial thromboplastin time (APTT) and the prothrombin time (PT) were determined using standard reagents and procedures from the Dade division of Baxter Healthcare. A whole blood clotting time (WBCT) was performed by diluting 0.1 mL of blood with 0.1 mL of saline and adding 0.1 mL of 0.02 mmol/L $CaCl_2$.

Platelet Aggregation

Male Sprague Dawley rats (350–450 g) and neutered male ferrets (1.1–1.3 kg) were anesthetized with Na-pentobarbital (50 and 45 mg/kg, i.v., respectively). Blood was sampled from catheters (PE-50) placed in the ferret femoral artery and in the rat carotid artery before and 1-hr after administration of aspirin at a dose of 10 mg/kg, i.v. These blood samples were drawn into anticoagulant (5 mL into a 1/10 final volume of 3.8% Na-citrate). Concentration-dependent platelet aggregation responses to collagen (Collagen reagent Horm; Hormon-Chemie, Müchen, Germany) were determined in whole blood using the standard impedance recording method described for a model 540 platelet aggregometer (Chrono-Log Corp, Havertown, PA).

Blood was also obtained in Na-citrate anticoagulant from human volunteers, ferrets, and rats that were not given the antiplatelet drugs. Blood samples were spun for 2.5 to 3 sec in the Microfuge E centrifuge to prepare platelet rich plasma (PRP) and again for 3 min to prepare platelet poor plasma (PPP). Platelet counts in PRP were adjusted to $\sim 200 \times 10^6/\mu L$ (human) or $\sim 350 \times 10^6/\mu L$ (ferret, rat) by PPP addition. Concentration-dependent responses of PRP to collagen, ADP (Sigma Chemical Co., St. Louis, MO) and U-46619 (BioMol Lab., Philadelphia, PA) were determined by the photometric technique described for the model 540 aggregometer.

Statistical Analysis

In the thrombosis studies drug effects relative to vehicle were determined by a *t* test for two-way comparisons, or an analysis of variance with Dunnett's for >two-way comparisons. Baseline CBF was added as a covariate in the total CBF analysis, and frequency of occlusion data were compared using the Fisher exact test. A repeated measures analysis of variance with contrasts was used to analyze hemodynamics and in ex vivo platelet aggregation studies. Analysis of variance with Tukey's test was used for the in vitro aggregation data. Computations were performed using Systat software and procedures (Evanston, IL). All data are presented as mean ± SEM. A $p < .05$ was considered significant.

Results

Antithrombotic Activity of Aspirin and Ifetroban

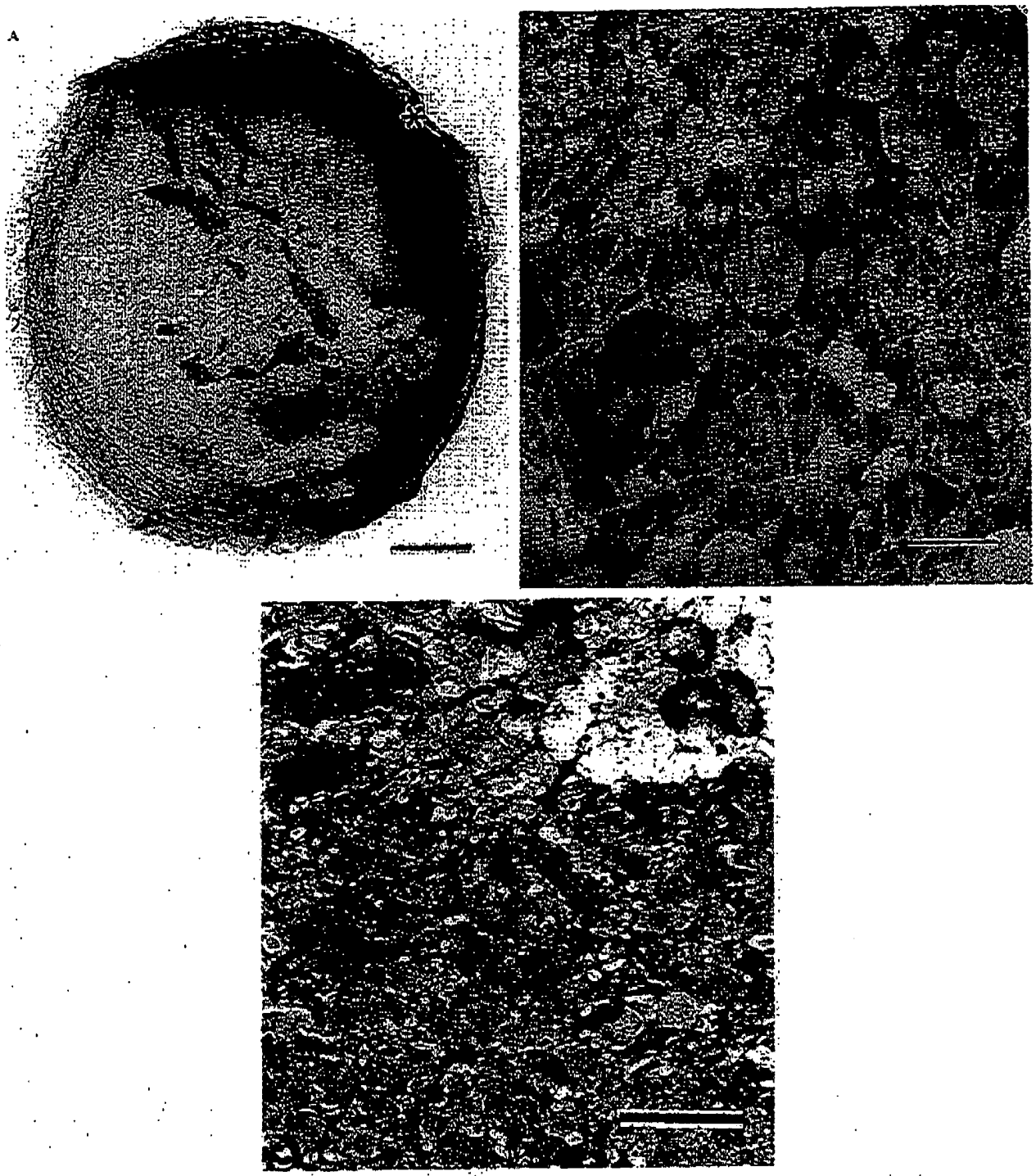
In preliminary experiments electrical stimulation at 0.5 mA produced occlusive thrombosis in 4/7 ferrets, which was considered inadequate. Average thrombus weight in these animals was 5.0 ± 1.0 mg ($n = 7$). Increasing the current to 1 mA resulted in a more reproducible and steady decline in CBF after termination of electrical stimulation. This is shown in the representative tracings in Figure 1. Occlusive thrombosis was observed in 7/7 vehicle-treated ferrets at the higher current with CBF decreasing from a baseline of 11.2 ± 0.9 to 0 mL/min in an average of 41 ± 3 min. These thrombi had an average weight of 8.1 ± 1.2 mg ($n = 7$), which was greater than that obtained with 0.5-mA stimulation ($p < .05$). Drug studies were completed using the 1-mA stimulation.

The approximate location of the electrode was readily discernible by light microscopy in vehicle-treated ferrets and was accompanied by a large well-developed thrombus that occluded the vascular lumen (Figure 2a). The thrombus was composed of numerous platelets enmeshed amongst a fibrin network as observed by scanning electron microscopy (Figure 2b). Variable numbers of erythrocytes were trapped within the thrombus, and their contour was occasionally distorted. Similar features were observed by transmission electron microscopy, with the addition of markedly swollen platelets having lost their secretory granules, and sparse numbers of trapped, degenerative leukocytes (Figure 2c).

Antithrombotic activity was identified by reductions in thrombus weight along with improvements in vessel patency and blood flow. The total CBF in each experiment was calculated by planimetry over the 3-hr observation period and was normalized as percent of control baseline flow (0 min). We found this to be a good method of quantitating CBF stability during thrombosis, and it also provided an index of average CBF over the entire experiment.

Aspirin decreased average thrombus weight 58% and improved average CBF, to the extent that vessel patency was maintained in all but one animal (Figure 3). The antithrombotic activity of ifetroban was dose-dependent. Ifetroban given at a dose of 0.3 mg/kg + 0.3 mg/kg per hr produced an insignificant 30% reduction in thrombus

JPM Vol. 35, No. 1
February 1996:3–10



approximate location of the electrode *(asterisk)* was easily discernible and was accompanied by a large well-developed thrombus that occluded the vascular lumen. Bar equals 200 μm. (B) Scanning electron micrograph in which the thrombus was composed of numerous platelets enmeshed among a fibrin network. A few erythrocytes are also present. Bar equals 5 μm. (C) Transmission electron micrograph in which the platelets were markedly swollen and have lost their secretory granules. These platelets were accompanied by fibrin aggregates and a degenerative leukocyte. Bar equals 5 μm.

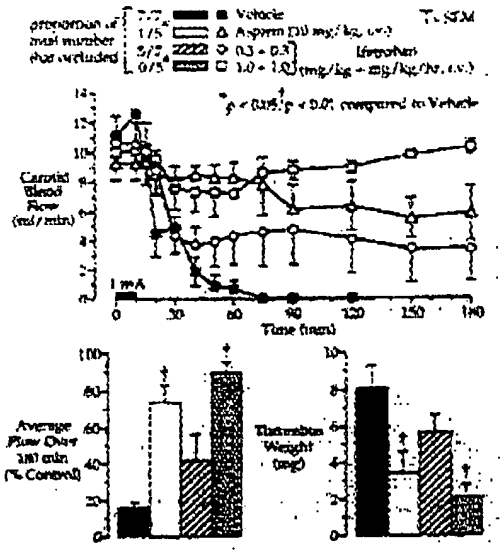


Figure 3. Changes in CBF, vessel patency, and thrombus weight were determined in ferrets subjected to electrical stimulation of the carotid artery. Significant differences relative to vehicle were detected using analysis of variance for average CBF and thrombus weight, and the Fisher exact test for vessel patency. Baseline (0 min) carotid blood flow did not differ significantly among the treatment groups. Aspirin and ifetroban were administered 15 min before electrical stimulation and had no direct effect on CBF (not shown). Thrombus weight tended to be lower in the high dose ifetroban compared to the aspirin group, but this difference was not significant.

weight without improving CBF or vessel patency. However, thrombus weight was decreased 74% and occlusion was prevented in all ferrets given a higher dose of 1 mg/kg + 1 mg/kg per her (Figure 3). Mean ABP did not change in any of the treatment groups during thrombus formation (Figure 4). ABP tended to be higher in the vehicle group throughout most of the experiment, but this difference was not significant. Carotid artery diameters (mm) did not differ between the vehicle (1.27 ± 0.04), aspirin (1.26 ± 0.18), and low (1.29 ± 0.04) or high (1.24 ± 0.04) dose ifetroban groups.

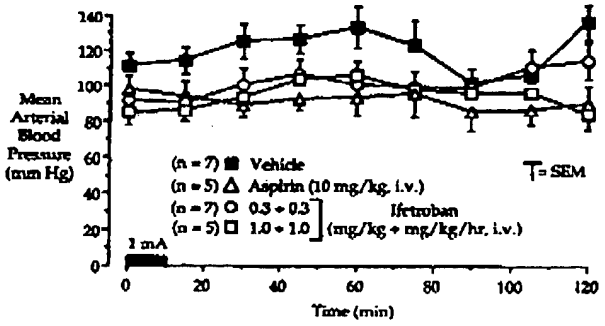


Figure 4. Changes in arterial blood pressure were determined in ferrets subjected to electrical stimulation of the carotid artery. There were no significant differences detected between or within treatment groups by analysis of variance with repeated measures.

The antithrombotic effect of aspirin and ifetroban was not accompanied by anticoagulant activity. The ex vivo indices of WBCT, APTT, or PT were not significantly prolonged in either vehicle- or drug-treated ferrets (Table 1). Baseline platelet counts in ferret blood ($631 \pm 51 \times 10^6/\mu L$, $n = 8$) were also not affected by treatment with aspirin ($598 \pm 45 \times 10^6/\mu L$, $n = 4$) or the high dose of ifetroban ($692 \pm 30 \times 10^6/\mu L$, $n = 4$).

Effect of Aspirin on Collagen-Induced Platelet Aggregation Determined Ex Vivo

Collagen was a more potent and powerful inducer of platelet aggregation in ferret compared to rat blood, and aspirin was a more effective platelet inhibitor in the ferret (Figure 5). In both species the ability of aspirin to inhibit collagen-induced platelet aggregation ex vivo could be surmounted by increasing collagen concentration. The concentration of collagen required for a 50% aggregation response (EC_{50}) in control samples was increased by aspirin to a greater extent in ferrets (0.9 ± 0.1 to 5.7 ± 0.8 μg/mL, $n = 5$) compared to rats (1.6 ± 0.2 to 3.1 ± 0.4 μg/mL, $n = 5$). The resulting concentration ratio for collagen activation (aspirin EC_{50}/Control EC_{50}, i.e., (aspirin EC_{50}/Control EC_{50}) was also greater in the ferret (6.9 ± 1.3) than in the rat platelet (2.0 ± 0.2, $p < .05$). Aggregation of rat and ferret platelets was unaffected by vehicle. These data refer to platelet activation as the maximum rate of aggregation (ohm/min). Similar drug effects were observed when aggregation was represented as magnitude of response (ohms), although there was more variability in this index

Table 1. Effect of Aspirin and Ifetroban on Ex Vivo Whole Blood Clotting Time (WBCT), Activated Partial Thromboplastin Time (APTT), and Prothrombin Time (PT) in Ferrets

Treatment	Sample	WBCT (sec)	APTT (sec)	PT (sec)
Vehicle ($n = 7$)	pre	84.6 ± 5.5	19.6 ± 0.6	12.0 ± 0.2
	post	66.5 ± 7.5*	20.7 ± 1.2	12.9 ± 0.5
Aspirin ($n = 6$)	pre	76.3 ± 7.1	19.4 ± 0.5	12.5 ± 0.6
(10 mg/kg)	post	76.0 ± 7.4	18.2 ± 0.6	12.6 ± 0.6
Ifetroban ($n = 4$)	pre	93.9 ± 3.7	18.7 ± 0.9	11.8 ± 0.1
(0.3 + 0.3[a]	post	89.8 ± 5.63	18.5 ± 0.6	11.7 ± 0.1
Ifetroban ($n = 5$)	pre	95.9 ± 5.76	20.2 ± 0.3	14.0 ± 0.8
(1 + 1)[a]	post	100.5 ± 3.3	19.6 ± 0.3	13.5 ± 0.9

Data are mean ± SEM.
[a] Dose given i.v. as a mg/kg injection + mg/kg per hr infusion.
* $p < .05$ comparing post-to pre-treatment value by paired t test.

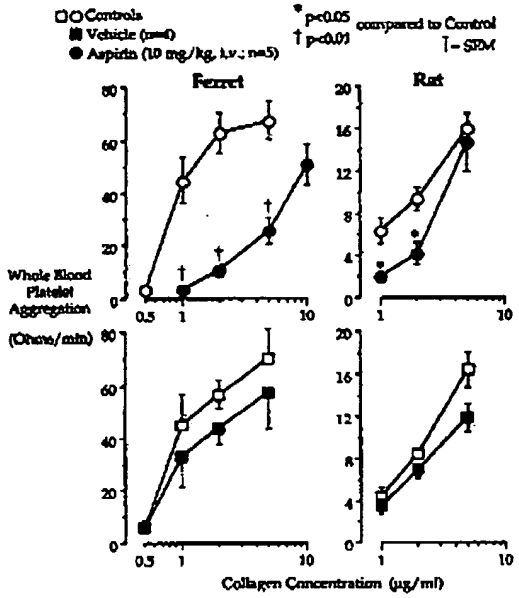


Figure 5. Whole-blood platelet aggregation responses to collagen were determined ex vivo before (control) and 1 hr after aspirin or vehicle dosing in anesthetized ferrets and rats. Significant differences between treatment and control responses were detected only in the aspirin group by analysis of variance with repeated measures.

(not shown). The maximum aggregation response to collagen (5 µg/mL) was also greater in ferrets (127 ± 10 ohms, $n = 4$) than in rats (51 ± 5 ohms, $n = 4$; $p < .05$).

Comparison of Platelet Aggregation Between Humans, Ferrets and Rats Determined in Vitro

There were profound differences in the in vitro reactivity of PRP obtained from humans, ferrets, and rats. There were no differences in maximum aggregation induced by collagen, but platelet aggregation in humans compared to the other species was greater with ADP, and even more so with U-46619 (Figure 6). Platelets from all 6 human volunteers aggregated >70% to U-46619, while 9 out of 10 rats produced a <20% response. The reactivity of ferret platelets was intermediate, with a <20% response to U-46619 observed in only 1 out of 9 ferrets. Variability to collagen resulted from differences in potency, with lower EC_{50}s obtained using platelets from humans (0.9 ± 0.2 µg/mL, $n = 6$) compared to ferrets (6.7 ± 2.5 µg/mL, $n = 5$; $p < .05$) or rats (20.5 ± 1.4 µg/mL, $n = 5$; $p < .05$). In contrast, EC_{50} values for ADP were similar in human (1.8 ± 0.2 µmol/L, $n = 6$), ferret (2.9 ± 0.2 µmol/L, $n = 5$), and rat (2.0 ± 0.2 µmol/L, $n = 5$) platelets. Human platelets responded to lower concentrations of U-46619 (EC_{50} = 1.1 ± 0.1 µmol/L, $n = 6$) relative to ferret platelets (EC_{50} = 10.1 ± 1.4 µmol/L, $n = 9$). The lack of platelet reactivity to U-46619 in rats precluded EC_{50} calculations. A platelet shape change response to U-46619 was observed in all of the species.

Discussion

Both similarities and differences were noted in the ferret and rat models of carotid artery thrombosis induced by external electrical stimulation. In vehicle-treated ferrets, this form of injury resulted in a high incidence of vascular occlusion with platelet- and fibrin-rich thrombi. Combining results from this study with other unpublished investigations, occlusive thrombosis has been observed in 16 out of 18 vehicle-treated ferrets. An identical stimulation of the rat carotid artery has produced occlusive thrombi in 97% of vehicle-treated rats with a morphology similar to that observed in the ferret (Schumacher et al., 1993b). In an overall comparison between the ferret ($n = 7$) and the rat ($n = 33$), control CBFs were higher (11.2 ± 0.9 v 4.4 ± 0.3

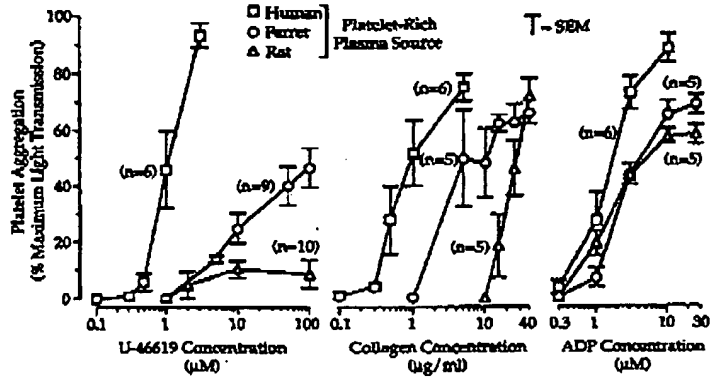


Figure 6. Aggregation responses of PRP derived from humans, ferrets, and rats were determined in vitro. Maximum aggregation responses were compared between species by analysis of variance. No differences were detected with collagen, while humans produced greater responses than ferrets or rats with U-46619 ($p < .01$) and ADP ($p < .05$). Significant differences in potency were also observed with U-46619 and collagen (see text).

mL/min), thrombus weights were greater (8.1 ± 1.1 v 3.9 ± 0.2 mg), and times to occlusion were longer (41 ± 3 v 31 ± 1 min) in the ferret. CBF decreased in a steady manner in both species and did not recover once occlusion was observed in any vehicle- or drug-treated animal. The difficulty of surgical preparation was similar for both species, although ferrets required mechanical ventilation with monitoring of blood gases to insure preparation stability. We studied ferrets one at a time, but they could be run in pairs as is usually done in the rat thrombosis model. Larger blood samples can be obtained from ferrets than rats, which is especially useful for ex vivo measurements of platelet function and coagulation before and after drug treatment. Preparations were maintained longer after initiating thrombosis in ferrets (180 min) than in rats (100 min) to accommodate the longer occlusion times and more extensive ex vivo studies conducted with ferrets.

The utility of the ferret model was further evidenced by the benefit of prophylactic treatment obtained with aspirin and ifetroban. Neither drug affected ex vivo blood coagulation, suggesting that antithrombotic activity resulted from platelet inhibition. Dose selection was based on ex vivo ferret studies in which aspirin (10 mg/kg) inhibited the TxA_2-generating capacity of blood by 99% (Gomoll and Ogletree, 1995), and ifetroban (0.3 mg/kg + 0.3 mg/kg per hr) blocked 99% of TxA_2 receptors on platelets (Gomoll et al., 1995). The dose of ifetroban required for efficacy was greater in the electrolytic compared to Folts model (Gomoll et al., 1995), which identifies the former as being more severe.

Reductions in thrombus weight were accompanied by improved CBF and vessel patency. A flow-limiting stenosis was not in place prior to vessel injury, and therefore CBF would not be expected to decline until a near occlusive thrombus had formed. In this situation a partially formed thrombus could be inhibited without measurable effects on CBF, so it is wise to include measurements of thrombus weight in this type of experiment.

The antithrombotic activity of aspirin and ifetroban was previously determined in the rat carotid artery subjected to a 0.5-mA (Schumacher et al., 1992) or 1.0-mA stimulation (Schumacher et al., 1993b). Ifetroban inhibited thrombosis induced by 1-mA stimulation to a slightly greater extent in the ferret (74%) compared to the rat (56%), although in ferrets this required a high pharmacological dose. In rats, a wide range of aspirin doses (1–30 mg/kg, i.v.) did not affect arterial thrombosis produced by the 1-mA injury, although a 10 mg/kg dose of aspirin was partially active against the 0.5-mA injury. This contrasts with the 58% thrombus inhibition achieved with aspirin in the ferret against 1-mA stimulation. Other investigators using rat models of arterial thrombosis have reported either no activity over a wide range of aspirin doses (Ashida et al., 1980; Massad et al., 1987), or inhibition only at aspirin doses of ~3–10 mg/kg (Parris et al., 1983; Philp et al., 1983).

To understand why aspirin was more antithrombotic in ferrets than rats, we determined the activity of aspirin against platelet aggregation responses to collagen in both species. Whole blood was used instead of PRP to better approximate in vivo conditions, and because erythrocytes have been shown to diminish aspirin's activity against platelets (Santos et al., 1991). Aspirin was a more effective antiplatelet drug ex vivo in ferrets than rats, and ferret platelets also mounted a larger response to collagen. This suggests that differences in platelet reactivity can contribute the disparate antithrombotic effects of aspirin. In human blood, Watts and coworkers (1991) reported a concentration ratio for aspirin (4.0 ± 0.1) that was intermediate to what we found in ferrets and rats (6.9 ± 1.3 and 2.0 ± 0.2 respectively).

Additional species comparisons were obtained using PRP and a more standard photometric technique. The ferret proved to be closer to the human than did the rat in these experiments. The variability in platelet reactivity depended upon the agonist used, with ADP being the least variable. Differences were observed in both potency (collagen, U-46619) and magnitude of response (ADP, U-46619). The failure of rat platelets to aggregate directly to U-46619 has been reported by others (Nakano et al., 1989), and is similar to what is observed with dog platelets under low calcium conditions (Burke et al., 1983). However, U-46619 induces a platelet shape change in both species, and we find that the antithrombotic activity of TxA_2 receptor antagonists correlates best with inhibition of this response (Schumacher et al., 1993a).

Our primary objective in establishing ferret thrombosis models was to complement a coronary artery occlusion-reperfusion model developed in this species (Gomoll and Lekich, 1990). Combination of antithrombotic and cardioprotective activities in a drug is desirable because vascular thrombosis usually precipitates acute myocardial infarction. It is best to evaluate both activities in a single species, and ferrets are one of the smaller and less expensive animals for this purpose. We have shown that aspirin and ifetroban are both antithrombotic in the ferret, while ifetroban, but not aspirin 10 mg/kg), is cardioprotective (Gomoll and Ogletree, 1995). This demonstrates how concurrent application of these experimental models can effectively differentiate antiplatelet drugs that share some pharmacological activities, such as thromboxane inhibition.

The excellent technical assistance provided by Sonia Youssef in the in vitro platelet aggregation studies is acknowledged.

JPM Vol. 35, No. 1
February 1996:3–10

References

Ashida S, Sakuma K, Abiko Y (1980) Antithrombotic effects of ticlopidine, acetylsalicylic acid and dipyridamole in vascular shunt model in rats. *Thromb Res* 17:663–671.

Burke SE, Leffer AM, Nicolaou KC, Smith GM, Smith JB (1983) Responsiveness of platelets and coronary arteries from different species to sythetic thromboxane and prostaglandin endoperoxide analogues. *Br J Pharmacol* 78:287–292.

Bush LR, Shebuski RJ (1990) In vivo models of arterial thrombosis and thrombolysis. *FASEB J* 4:3087–3098.

Gomoll AW, Lekich RP (1990) Use of the ferret for a myocardial ischemia/salvage model. *J Pharmacol Methods* 23:213–223.

Gomoll AW, Ogletree ML (1995) Failure of aspirin to interfere with the cardioprotective effects of ifetroban. *Eur J Pharmacol* 271:471–479.

Gomoll AW, Schumacher WA, Ogletree ML (1995) Dose-related cardioprotection by ifetroban in relation to inhibition of ex vivo platelet function and thrombosis. *Pharmacology* 50:92–110.

Hladovec J (1973) Experimental arterial thrombosis in rats with continuous registration. *Thromb Diath Haemorrh* 29:407–410.

Massad L, Plotkine M, Capdeville C, Boulu RG (1987) Electrically induced arterial thrombosis model in the conscious rat. *Thromb Res* 48:1–10.

McDowell EM, Trump BF (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 100:405–414.

Nakano T, Hanasaki K, Arita H (1989) Role of protein kinase C in U46619-induced platelet shape change, aggregation and secretion. *Thromb Res* 56:299–306.

Ogletree ML, Harris DN, Schumacher WA, Webb, Misra RN (1993). Pharmacological profile of BMS 180,291:A potent, long-acting, orally active thromboxane A_2/prostaglandin endoperoxide receptor antagonist. *J Pharmacol Exp Ther* 264:570–578.

Parris J, Fournau P, Granero M, Veins C (1983) Antithrombotic effect of very low doses of acetylsalicyclic acid in rats. *Thromb Res* 28:313–321.

Philp RB, Paul ML, Killackey JJ, Killackey BA (1983) The influence of dose, time of administration, body temperature and salicylate kinetics on the antithrombotic action of acetylsalicylic acid in male rats. *Haemostasis* 13:42–52.

Romson JL, Haack DW, Lucchesi BR (1980) Electrical induction of coronary artery thrombosis in the ambulatory canine: A model for in vivo evaluation of anti-thrombotic drugs. *Thromb Res* 17:841–853.

Salazar AE (1961) Experimental myocardial infarction, induction of coronary thrombosis in the intact closed-chest dog. *Circ Res* 9:1351–1356.

Santos MT, Valles J, Marcus AJ, Safier LB, Broekman MJ, Islam N, Ullman HL, Eiroa AM, Aznar J (1991) Enhancement of platelet reactivity and modulation of eicosanoid production by intact erythrocytes. *J Clin Invest* 87:571–580.

Schumacher WA, Heran CL (1989) Effect of thromboxane antagonism on recanalization during streptokinase-induced thrombolysis in anesthetized monkeys. *J Cardiovasc Pharmacol* 13:853–861.

Schumacher WA, Heran CL, Steinbacher TE, Megill JR, Bird JE, Giancarli MR, Durham SK (1992) Thrombin inhibition compared with other antithrombotic drugs in rats. *Thromb Res* 68:157–166.

Schumacher WA, Heran CL, Steinbacher TE, Youssef S, Ogletree ML (1993a) Superior activity of a thromboxane receptor antagonist compared to aspirin in experimental arterial and venous thrombosis. *J Cardiovasc Pharmacol* 22:526–533.

Schumacher WA, Steinbacher TE, Heran CL, Megill JR, Durham SK (1993b) Effects of antithrombotic drugs in a rat model of aspirin-insensitive arterial thrombosis. *Thromb Haemost* 69:509–514.

Van der Giessen WJ (1989) The effect of the thromboxane receptor antagonist BM 13,177 on experimentally induced coronary artery thrombosis in the pig. *Eur J Pharmacol* 147:241–248.

Watts IS, Wharton KA, White BP, Lumley P (1991) Thromboxane (Tx) A_2 receptor blockade and TxA_2 synthase inhibition alone and in combination: Comparison of anti-aggregatory efficacy in human platelets. *Br J Pharmacol* 102:497–505.

Zweiler AJ (1967) Impairment of platelet function and thrombus growth in reserpine-treated rabbits. *J Lab Clin Med* 70:16–20.